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Alternative ingredients in calf milk replacer – a review for bovine practitioners

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Abstract

Non-dairy source ingredients for calf milk replacer formulations have been evaluated in North America and Europe, and are routinely used in European milk replacer formulas. Dairy origin ingredients are increasingly less economical for use in animal feed due to increased global demand for these high-quality foodstuffs. As an alternative, specific non-dairy ingredients are satisfactory for neonatal calf diets when properly incorporated into calf milk replacers. Plant-sourced proteins and oils must be properly processed for efficient utilization. Feeding spray-dried bovine plasma results in calf performance comparable to feeding milk protein when incorporated at recommended levels in calf milk replacers, and it reduces calf morbidity and mortality. Replacing milk lactose is more problematic because pre-ruminant calves lack intestinal enzymatic activity to digest plant-based polysaccharides. The objective of this review is to provide cattle veterinarians detailed information about alternative ingredients that are acceptable for formulation into calf milk replacers.

Key words: calf milk replacers, alternative ingredients, spray-dried bovine plasma, amino acid profiles, carbohydrate and lipid digestibility

Résumé

Les laits de remplacement pour les veaux contenant des ingrédients autres que laitiers ont été évaluées en Amérique du Nord et en Europe et sont utilisées de façon routinière dans le lait de remplacement en Europe. Il est de moins en moins avantageux économiquement d'utiliser des ingrédients laitiers dans l'alimentation des animaux en raison de la demande mondiale accrue pour ces ingrédients d'alimentation de haute qualité. En tant qu'alternative, des ingrédients non laitiers spécifiques peuvent satisfaire le régime alimentaire des veaux nouveau-nés lorsqu'ils sont correctement intégrés dans le lait de remplacement des veaux. Les protéines et huiles d'origine végétale doivent être convenablement traitées pour une utilisation efficace. Au niveau de la performance des veaux, la prise alimentaire de plasma bovin séché par pulvérisation équivaut à la prise alimentaire de protéines laitières si elle est intégrée suivant les règles établies dans le lait de remplacement des veaux. Elle peut aussi réduire la morbidité et la mortalité. Il est plus difficile de remplacer le lactose laitier parce que les veaux non ruminants n'ont pas l'activité enzymatique intestinale nécessaire pour digérer les polysaccharides d'origine végétale. L'objectif de ce survol est de fournir de l'information détaillée aux vétérinaires de bovins concernant les ingrédients alternatifs qui sont acceptables dans la formulation du lait de remplacement des veaux.

Introduction

Whey and milk proteins are commonly used as protein ingredient sources for calf milk replacer (MR) formulation. Human demand for dairy proteins has resulted in significant competition for these high-quality proteins. Consequently, European Economic Community countries routinely manufacture calf MR utilizing a number of non-whey proteins, as well as alternatives to animal fat. Limited replacement of lactose is practiced in Europe as well. An infrastructure has developed to provide alternative ingredients for MR manufacturing in the European calf MR market, which supports a non-veal calf MR industry that is 4 times as large as the US, and veal numbers that overshadow US production 18-fold.[5,38] There is significant use of alternative protein, fat, and carbohydrate sources in veal and calf MR formulas.

This review examines research where nutrient sources other than milk-based ingredients were evaluated in calves fed MR. References are from peer-reviewed journals except for some reference books, symposia proceedings, 23 abstracts identified in the list of references, and 1 dairy magazine article. Published data represents both newborn and veal calves of varying ages. Studies evaluating alternative MR nutrients in accelerated feeding programs are sparse. A review of the physiology of the calf digestive tract is provided where applicable. The purpose of this review is to provide bovine practitioners information to assist their calf-raising clients in making informed decisions about which alternative ingredients marketed by calf MR manufacturers produce comparable calf performance to that of milk-sourced ingredients.

Sample sizes are listed in the tables for experiments that reported results for hydrolyzed (soluble) wheat gluten protein, various soy proteins, spray-died bovine plasma, and egg proteins. Statistical testing in trials was not always similar. The authors of the present paper have listed those values in the text considered important to interpretation of outcomes, with trends being $P \leq 0.10$, significant being $P \leq 0.05$, and highly significant declared at $P \leq 0.01$. The significance values are reported as presented in papers reviewed.

Plant Protein Sources

Challenges with Plant-Based Proteins in Calf Milk Replacers

It has been postulated that reduced digestibility of plant proteins,[97] resulting in reduced calf performance, is multifactorial. Some postulate that reduced calf performance is due to lower intrinsic digestibility of large, complex plant proteins, reducing or resisting digestive enzyme hydrolysis,[122] while other research points to higher losses or passage of endogenous proteins.[21,99] Anti-nutritional factors in plant protein matrices, such as antigenic proteins, tannins, lectins, or trypsin inhibitors, have been suggested as causes of intestinal or mucosal modification of enzyme hydrolysis, potentially reducing or impairing absorptive capacity of the intestinal tract.[41,106,117] A 2003 veal calf study[112] determined increasing dietary crude protein (CP) in the MR diet, regardless of protein source, increased flow of oligopeptides and free amino acids in ileal digesta. When plant proteins replaced a portion of the MR diet's CP compared to MR formulated with skim milk protein, accumulation of peptides, oligopeptides, and free amino acids resulted in ileal digesta, indicating less digestion and absorption occurred compared to skim milk proteins. A study in rats led to postulation that accumulation of undigested peptides saturated absorption sites responsible for transport of amino acids and di- and tri-peptides across the intestinal mucosal barrier, blocking amino acid uptake.[202] Increased secretion of endogenous proteins was partially explained by increased mucin secretion and bacterial protein complexes.[115,116,125,126,156]

A veal study[112] reported an increase in flow of proteins that have a molecular weight greater than 20,000 in ileal digesta of calves fed MR diets with partially hydrolyzed soybean protein isolate or soybean protein concentrate, when compared to MR diets formulated with skim milk powder.

Milk proteins enter the duodenum largely intact, become partially degraded in the jejunum, and complete their digestion processes in the ileum.[32] Others reported that the flow of CP with a molecular weight of less than 400 significantly increased as skim milk-based CP increased in MR diets.[114] Comparably, digestion-resistant dietary protein fractions have been found in ileal digesta of calves fed soybean or potato proteins,[19,94,95,100] pigs fed kidney bean protein,[9] and broilers fed pea protein.[42]

Three MR were evaluated in a single 8-week-old veal calf study: 1) CP provided exclusively by skim-milk powder; 2) skim-milk powder with 52% CP substituted with native wheat gluten; and 3) 52% of CP provided as potato-protein concentrate.[19] Utilizing immunochemical detection, protein fractions with a molecular weight of 43,000 and below 14,000 were detected in ileal digesta in calves consuming potato-protein concentrate, but no immunoreactivity was discovered in ileal digesta taken from calves consuming the native wheat-gluten diet. Fecal nitrogen digestibility testing determined potato protein concentrate was less digestible than native wheat gluten, 0.90 vs 0.93, and both alternative protein sources were less digestible than skim-milk protein, (0.95; $P < 0.05$). Undigested amino acids recovered at the distal ileum in this evaluation were always greater for gluten and potato proteins than for skim-milk protein; potato protein is not used in the US MR market.[44,127] Solubilized or hydrolyzed wheat gluten has high digestibility at both fecal[180] and ileal[22,186] sites, but this trial examined wheat-gluten protein that was not hydrolyzed. Native wheat gluten retains viscoelastic properties, which may limit digestibility.

Hydrolyzed (Soluble) Wheat Gluten Protein (SWGP)

Soluble wheat-gluten protein (SWGP) is derived from wheat flour. Non-hydrolyzed wheat gluten is 1 of the largest proteins in nature and strongly repels water. A mixture of wheat gluten and water literally has chewing gum-like properties. Wheat gluten is highly digestible in baby pig feeds,[152] but its large particle size renders it insoluble in MR. The SWGP is manufactured by moistening wheat flour to make dough, then using water to extract soluble proteins and starch, and isolating gluten. Manufacturers expose gluten to enzymes that hydrolyze the protein into smaller molecular weights, and mechanical procedures are used to further extract starch-containing water. Caution is used to stop enzymatic hydrolysis at optimal time and temperature exposure. Too much hydrolysis results in bitterness and palatability issues, while insufficient hydrolysis results in sub-optimal solubility and digestibility. Heat is utilized to stop enzymatic action. The end product is then spray-dried for commercial use in MR diets.[195]

Soluble wheat-gluten protein sources are 78 to 87% CP (as-is basis) (Figure 1) and 1 to 3% ash. Properly hydrolyzed SWGP is a cream-colored powder with neutral taste and no odor, and does not change the color or appearance of reconstituted MR or result in sediment. Appropriate American Association of Feed Control Officials definition of SWGP is "hydrolyzed wheat gluten". Typical SWGP inclusion rate is 5% (100 lb (45.4 kg)/ton of MR powder), replacing approximately 20% of the milk-based protein in the formula.

European veal calf research conducted in 1991 on 176.4 lb (80 kg), 8.5-week-old Dutch Friesian x Holstein Friesian calves demonstrated SWGP could replace approximately 28.5% or 47.3% of milk-sourced proteins in a liquid

Figure 1. Nutrior hydrolyzed wheat-gluten protein, 84% crude protein. Manufactured by Chamtour, Bazancourt Cedex, France.

veal formula, resulting in the same fecal digestibility as a skim-milk-based diet.[180] Two subsequent European veal calf research trials showed slightly lower digestibility for SWGP-based formulas as compared to skim-milk-based formulas. The first study of ileal digestibility of a MR formula containing 75% SWGP, 21% whey, and 4% synthetic lysine was done by administering it via an abomasal catheter to 2-month-old calves. The control diet contained 100% skim milk and resulted in digestibility that tended ($P = 0.08$) to be greater, 93% versus 87%, for skim- vs SWGP-based diets, respectively.[22] The second veal calf trial compared digestibility of MR administered via abomasal catheter and composed completely of either milk protein (control) or MR composed of either 24% or 76% of the CP from SWGP. Ileocecal cannulated calves were, on average, 306 lb (139 kg) and 85 days of age. They were switched between the 3 diets every 2 weeks over a 6-week period. Apparent ileal digestibility of nitrogen was 91%, 89% ($P < 0.05$), and 85% ($P < 0.01$ vs control, and $P < 0.05$ vs moderate SWGP) for the skim milk-based, moderate SWGP- and high SWGP-based diets, respectively.[186] Other US research also was conducted on dairy calves to examine the replacement of 50% of the milk protein.[172] Numerical, but not statistically significant, lower 14 and 42-day average daily gain (ADG) resulted for both of 2 different sources of SWGP vs the all-milk formula. Another study compared feeding 20:20 MR (20% CP and 20% crude fat on an as-fed basis, 1.2 lb (0.54 kg)/calf/day) composed of all-milk protein or either 7.5% (30% of CP) or 12.5% (50% of CP) SWGP and an 18:20 MR composed of either all-milk protein or 7.4% (33% of CP) SWGP.[178] There were no differences in 2, 4, or 6-week weight gain of calves in any treatment, with the exception of the 18:20 SWGP formula at 4 weeks of age. Calves fed this SWGP formula had higher total gain in the 2 to 4 wk feeding period (11.5 lb; 5.2 kg) compared to the all-milk protein MR (8.6 lb; 3.9 kg) by 2.9 lb (1.3 kg) ($P < 0.1$). Calves fed the 18:20 MR with SWGP had numerically higher gain at 42 days compared to their all-milk peers by 6.2 lb (2.8 kg) (23.4 vs 17.2 (10.6 kg vs 7.8 kg), respectively). Both trials examined SWGP in traditional, low-volume milk feeding regimens (2 quarts (1.9L) twice daily, 1.2 lb (0.54 kg) MR powder/d). Two more trials conducted in the 1990s showed SWGP (10% and 20% of CP) fed in MR formulas that also contained soy-protein concentrate (SPC, 40% and 50% of CP)[181] and SWGP fed in MR formulas with and without 0.1% addition of protease enzymes,[192] performed comparably to all-milk formulas (Table 1).

A French veal-calf study at the INRA/ENSAR Institute (French National Institute for Agriculture/Department of Social Services, Agriculture and Food, and the Environment) in Rennes evaluated SWGP when replacing 49% and 61% of the MR CP up to days 83 and 146 (market), respectively. The control diet was skim-milk powder-based.[128] Calves were 37 to 39 days old and 133 to 137 lb (60.33 to 62.14 kg) at the beginning of trial. SWGP formulas were balanced, using synthetic amino acids, to provide identical lysine, methionine, and threonine as skim-milk protein formulas. A SWGP group was fed a formula that balanced branched-chain amino acid levels of the 12.5% SWGP formula to levels of these amino acids found in the skim-milk protein formula. Synthetic valine, isoleucine, and leucine were used in addition to the 3 aforementioned synthetic amino acids. Feed allowances were set at 95% of ad libitum levels, and calves were allowed to reach a carcass weight close to 287 lb (130.2 kg) at 140 days. Allowances were adjusted every 3 days. The researchers found performance of calves fed SWGP fortified with branched-chain amino acids to be similar to calves fed the otherwise-equal SWGP (without synthetic branched-chain amino acids) or skim-milk (control) formula. This trial was conducted in 2002 after makers of SWGP most likely improved product consistency and quality in comparison to earlier trials, and may have incorporated a more appropriate synthetic amino acid fortification.

Research conducted on Holstein heifer calves compared a 20:20 MR that contained either 33% of CP from SWGP or an all-milk formula. No differences in calf health or in 42-day (weaning) ADG, starter grain intake or 56-day ADG were noted.[34] Holstein bull calves that were 10 ± 4 days of age and fed 22:18 MR with 50% of CP from SWGP performed comparably to calves fed the same quantity of all-milk MR that was 20:20.[35] Both trials were conducted using traditional 1.25 lb (0.57 kg)/calf/day MR feeding rates. These trials indicate calves fed SWGP-containing MR perform comparably to those fed an all-milk formula.

Research was performed on 2 to 4-day-old dairy heifers to compare an all-milk protein formula to SWGP replacing 50%, SWGP replacing 30%, soy-protein concentrate (SPC) replacing 50% or a combination of SWGP and SPC each replacing 25% of the milk protein.[69] All replacement strategies

Table 1. Published results of feeding hydrolyzed (soluble) wheat gluten protein (SWGP) to calves.

Reference (chronological order)	Diet CP & fat, %	% of total MR CP as Soluble Wheat Gluten Protein (SWGP)	ADG, lb/d	Scour score or feed/ gain for [128] or 14 d ADG for [172,192]	Death loss %	Starter Intake lb/ day	Age of calves, d
Ziegler D. *J Dairy Sci* 2014; 97 Abstract[203] 105 calves randomly assigned 4 groups 47.2 lb MR, 1.25 lb/d to 35 d, 0.625 lb/d to wean Wean 42 d. Calf starter 18% CP	20% CP 20% Fat	All-milk 50% CP from SWGP & plasma 50% CP from SPC & plasma 50% CP from SPC, SWGP & Plasma	1.432 1.320 1.278 1.300	1.37 (a) 1.30 (b) 1.25 (b) 1.26 (b)	- - - -	1.27 1.12 1.14 1.14	1 - 42 1 - 42 1 - 42 1 - 42
Hwang G. *J Dairy Sci* 2013; 96 Abstract[79] 20 calves per treatment; 21% CP starter All diets contained 36% skim milk protein 108 lb CMR fed over 56 days, step-up/ step down Diets B, C & D Cornell a.a. levels. E only lys, meth, thr	28.5% CP 15% Fat	A. All-milk (AM) (2.6% lysine) B. All-milk + amino acids (2.62% lys) C. 21% SWGP + a. a. (2.57% lys) D. 42% SWGP + a.a. (2.32% lys) E. 42% SWGP + a.a. (2.32% lys)	1.433 1.455 1.455 1.390 1.345	- - - - -	0 0 0 0 0	2.49 (a) 2.07 (b) 2.34 (a) 2.18 (a) 1.87 (a)	1 - 56 1 - 56 1 - 56 1 - 56 1 - 56
Chester-Jones. *J Dairy Sci* 2013; 96 Abstract[34] 28 calves all-milk, 27 SWGP. 46.7 lb CMR/calf	20% CP 20% Fat	All-mik 33% SWGP	1.170 1.120	1.07 1.04	- -	0.893 0.85	1 - 42 1 - 42
Wood D. *J Dairy Sci* 2009; 92 Abstract[196] All-milk 44 calves, wheat/plasma 42 calves 57.9 lb MR/calf; 17% CP starter grain	22% CP 20% Fat	All-millk 17.5% plasma & 24.5% SWGP	1.410 1.440	- -	10% 7%	- -	1 - 42 1 - 42
Carlson D. *J Dairy Sci* 2009; 92 Abstract[30] 102 calves randomly assigned 4 groups All-milk Control: 1.25 lb/d 1-35, 0.6 lb/d 36-42 Step-down: 1.25/d 1 - 14, 1/d 15-35, 0.5 36-42	20% CP 20% Fat	All-milk Control All-milk, Step-down Plasma, Step-down SWGP/Plasma, Step-down	1.257 1.322 1.300 1.190	5.25 (a) (days) 3.86 (b) 4.02 (b) 3.8 (b)	- - - -	1.41 (a) 1.74 (b) 1.65 (b) 1.61 (b)	1 - 56 1 - 56 1 - 56 1 - 56
Chestnut A. *J Dairy Sci* 2008; 91 Abstract[35] 36 calves all-milk, 34 SWGP. 39.4 lb CMR/calf	22:20 22:18	All-Milk Control 50% SWGP	1.158 1.215	- -	- -	1.23 1.44*	1 - 35 1 - 35
Hill TM. *Prof Anim Sci* 2008; 2424:465[72] (16 bull calves/treatment) 40.5 lb CMR/ calf	20% CP 20% Fat	All-milk 15% SWGP	1.222 1.051	4 d scouring 4.9 d scouring	0 0	1.352 1.132	1 - 42 1 - 42
Hill TM. *Prof Anim Sci* 2008; 2424:465[72] (16 bull calves/treatment) 18.5% CP starter	26% CP 17% Fat	All-milk 19% SWGP 38% SWGP	1.411 (a)*** 1.237 (b)*** 1.113 (c)***	10.8 d scouring 9 d scouring 11.1 d scouring	0 0 0	0.99 0.805 0.836	1 - 42 1 - 42 1 - 42
Hayes S. *J Dairy Sci* 2007; 90 Abstract[69] (124 calves randomly assigned 5 groups) 48.1 lb MR/calf; 20.2% CP starter grain Abstract states ADG were lower for alt. proteins due to reduced grain intake (intake not reported)	20% CP 20% Fat	All-millk 50% SWGP 50% Soy Protein Conc. (SPC) 30% SWGP 25% SWGP & 25% SPC	1.72 (a) 1.565 (b) 1.543 (b) 1.521 (b) 1.499 (b)	- - - - -	- - - - -	- - - - -	1 - 42 1 - 42 1 - 42 1 - 42 1 - 42
Ortigues-Marty I. *Reprod Nutr Dev* 2003; 43:57[128] (14 veal calves/treatment) Veal grower MR fed day 29-83 Veal finisher MR fed day 84-146 No grain fed at any time	22% CP 19% Fat 21% CP 21% Fat	All-milk 49% SWGP (+ lys., meth, threonine) 49% SWGP (+ lys, meth, thr) + BCAA^ All-milk 61% SWGP (+ lys, meth, thr) 61% SWGP (+ lys, meth, thr) + BCAA^	2.680 2.720 2.720 3.340 3.270 3.360	1.38 feed/gain 1.40 feed/gain 1.40 feed/gain 1.88 feed/gain 1.96 feed/gain 1.91 feed/gain	- - - - - -	0 0 0 0 0 0	29-83 29-83 29-83 84-146 84-146 84-146
Waterman D. *J Dairy Sci* 1997; 80 Abstract[192] (30 calves/treatment) Enzyme used: Fungal Protease 93 41.6 lb MR/calf; calf starter ad lib d 3 onward Feed conversion did not differ ($P < 0.10$)	20% CP 20% Fat	All-milk SPC (% not shown) SPC + 0.1% enzymes SWGP (% not shown) SWGP + 0.1% enzymes	0.510 0.419 0.463 0.507 0.551	0.242 14 d ADG 0.198 14 d ADG 0.220 14 d ADG 0.264 14 d ADG 0.33 14 d ADG	- - - - -	- - - - -	1 - 42 1 - 42 1 - 42 1 - 42 1 - 42

Toullec R. *Anim Feed Sci and Tech* 1997; 73[186] Ileal dig. study, INRA, Rennes cedex, France 306 lb BW, 6 cannulated calves switched every 2 wks	22% CP 21% Fat	All-milk 9% SWGP (24% of CP) 17.9% SWGP (76% of CP)	91% app. illeal digestibility of nitrogen (a) 89% app. Illeal digestibility of nitrogen (b) 85% app. Illeal dig. of nitrogen (****)			no grain no grain no grain	85 - 127 85 - 127 85 - 127
Terui H. *J Dairy Sci* 1996; 79:1261[178] (24 calves/treatment) 16% CP starter Starter grain introduced day 21 Starter intake is avg lb day 21 - 42	20% CP 20% Fat 18% CP 20% Fat	All-milk 30% SWGP 50% SWGP All-milk 33% SWGP	0.567 (ab) 0.53 (ab) 0.588 (a) 0.409 (b) 0.556 (ab)	- - - - -	4.20% 4.20% 4.20% 8.40% 0%	0.5 (a) 0.451 (ab) 0.462 (ab) 0.357 (b) 0.472 (ab)	1 - 42 1 - 42 1 - 42 1 - 42 1 - 42
Tomkins T. *J Dairy Sci* 1994; 77 Abstract[181] (240 calves randomly assigned 8 groups) Mortality column are morbidity/ removed 50 lb MR/calf; C.P. & Fat not reported starter grain intake day 15 - 56 Abstract reports no difference in health Day 1 - 14 ADG was superior ($P < 0.05$) for all-milk vs treatments C, D or H	- -	(A) All-milk (B) 50% SPC (C) 50% SPC, 10% SWGP (D) 50% SPC, 20% SWGP (E) 40% SPC, 10% SWGP (F) 40% SPC, 20% SWGP (G) 50% SPC, 10% Plasma (H) 50% SPC, 10% SWGP, 10% Plasma	0.882 0.960 0.871 0.780 0.800 1.070 0.860 0.811	- - - - - - - -	0.0% 6.7% 6.7% 13.3% 3.3% 10.0% 10.0% 20.0%	1.398 1.61 1.446 1.387 1.382 1.882 1.516 1.403	1 - 56 1 - 56 1 - 56 1 - 56 1 - 56 1 - 56 1 - 56 1 - 56
Sowinski J. *J Anim Sci* 1993; 71 Abstract[172] (30 calves/treatment)	20% CP 20% Fat	All-milk 50% SWGP brand #1 50% SWGP brand #2 50% SPC	1.120 0.980 0.950 1.040	0.43 14 d ADG 0.4 14 d ADG 0.35 14d ADG 0.35 14d ADG	- - - -	- - - -	1 - 42 1 - 42 1 - 42 1 - 42
Bush RS. *Ann Zootech* 1992; 41:31-32[22] (no grain) Ileal dig. study INRA, France. 100% MR, 3 calves		MR: 75% SWGP, 21% whey, 4% lysine 100% skim milk powder	87%* total nitrogen dosed a.a. ileal digestibility 93% total nitrogen dosed a.a. ileal digestibility				60 - 64 60 - 64
Tolman G. *EAAP Pub* 1991; 52:227[180] Fecal dig. study. ILOB-TNO, Wageningen, NL App. 176.4 lb body weight (5 calves/ treatment)	22.5:17# 28:15.8# 33.8:14.8#	All-milk 28.5% SWGP 47.33% SWGP	94.1% CP fecal digestibility 95.3% CP fecal digestibility 94.9% CP fecal digestibility			no grain no grain no grain	56 - 61 56 - 61 56 - 61

Subscripts different denotes $P < 0.05$
a.a. denotes synthetic amino acids (lysine, methionine, leucine, valine, isoleucine, threonine, tryptophan) added to balance same amino acid profile across diets (only in Hwang, 2013)
*** denotes $P < 0.01$ and *$P < 0.10$
**** denotes $P < 0.01$ from control and $P < 0.05$ from other SWGP diet
#denotes crude protein %: crude fat % content of MR
^BCAA denotes branched chain amino acids (added valine, isoleucine, leucine)

for milk protein performed comparably, but all gained less ($P < 0.05$) than the all-milk protein control formulas. Calves fed all-milk control formula gained 9 lb (4.1 kg) more than calves fed alternative protein formulas. Calves were fed 48 lb (21.8 kg) of 20:20 medicated MR over 42 days. Control calves had a 56-day ADG of 1.72 lb (0.78 kg). ADG for calves fed various alternative protein-based formulas ranged between 1.50 to 1.57 lb (0.68 to 0.71 kg).

An all-milk protein formula was compared to a 4.3% (15% of CP) inclusion of SWGP in a 1 lb (0.45 kg)/day 20:20 MR. Calves fed SWGP gained 20% less over 42 days ($P < 0.01$), consumed 16% less starter grain, and had significantly poorer feed conversion and hip width.[72] In a second trial, an all-milk protein formula was compared to 6% (19% of CP) and 12% (38% of CP) SWGP in 26:17 MR fed at 1.5 lb (0.68 kg)/day. A linear reduction in ADG and feed efficiency occurred with increasing concentrations of SWGP ($P < 0.01$). Calves fed MR containing 12% SWGP had a 21% lower ADG compared to those fed the all-milk protein control ($P < 0.01$).[72] These data, when compared to the data collected for 8-week-old veal calves,[180] clearly demonstrate that older calves digest plant proteins more efficiently.

All-milk, animal plasma, or animal plasma in conjunction with SWGP formulas were compared in Holstein heifer calves that were 2 to 4-days-of-age and fed the respective formula for 42 days.[30] No performance or health differences were noted. A later study reported that a combination of 6% SWGP and 5% plasma effectively replaced up to 42% of milk protein in calf MR, with no effect on 43-day gain in auction-sourced Holstein calves.[196] Calves fed wheat/plasma diets gained less at day 15 ($P < 0.023$) and more ($P < 0.049$) between days 29 and 43, perhaps exposing areas of weakness (first week or 2) and strength (period of highest starter grain intake) for SWGP. These 2 trials indicated that the inclusion of plasma with SWGP helped to offset the reduced performance in SWGP formulas. Low daily feeding rates of MR (1 lb (0.45

kg) MR powder) were followed in both trials. In addition to the 2 aforementioned trials, 2 more trials[181,203] showed that a combination of plasma and SWGP performed comparably to an all-milk MR.

To take advantage of alternative plant-sourced proteins formulated into calf MR, proper colostrum administration and management may be important. Research examining fatty acid and vitamin absorption in the first 7 days of a calf's life revealed delaying colostrum administration by 24 hours results in significantly impaired absorption of nutrients.[17] Research also demonstrates intestinal glucose absorption is impaired if colostrum is not fed.[174] Provision of colostrum not only results in improved epithelial tissue growth and maturation along the digestive tract, but also increases the quantity of pancreatic enzymes produced and their digestive activity.[16] Additional research is needed to quantify the impact of proper colostrum provision on calf digestibility of plant-sourced proteins in MR.

Amino acid supplementation is necessary to efficiently utilize SWGP in MR. Wheat protein is deficient in lysine compared to milk protein. To optimize performance, care must be taken to include synthetic lysine in SWGP-containing formulas. Threonine is about 40% lower in SWGP compared to dried skim-milk, and even lower in comparison to whey protein concentrate (WPC). Threonine is typically economical to incorporate into formulas.

While data demonstrated SWGP could perform well in calf MR,[34,35,79,128,172,178,180,192,196] some trials demonstrated poorer performance[30,69,72] when compared to an all-milk protein MR. We believe that inclusion of plasma protein mitigates reduced performance concerns. We recommend conservative use of SWGP at inclusion rates up to 5% of MR – particularly in conjunction with plasma – during the entire milk-feeding period, or alone in a 2-stage MR program.

Soy Protein

If properly processed, calf research indicates soy-based proteins (Figure 2) can perform comparably to milk proteins, but care must be taken to select only performance-proven soy sources. There are 3 common sources of soy protein used in calf milk replacers: soy flour (SF), soy-protein concentrate (SPC), and soy isolate (SI). SF is manufactured from de-fatted soybeans, typically hexane-extracted, toasted, and finely ground. Some manufacturers also enzymatically hydrolyze processed SF. SF is beige to dark beige and is typically 50% CP. SPC is processed to remove soluble carbohydrates and is higher in CP, typically 56 to 82% CP, depending upon the manufacturing process. An aqueous alcohol leach is a common method used to extract carbohydrates from SPC. Some SPC brands are enzymatically hydrolyzed, and all should be pulverized into a fine powder. SPC is cream to beige in color. Soy isolate, a soy protein with the carbohydrate fraction further reduced, is an off-white to beige fine powder.[195]

In considering how soy should be processed for use in MR, data regarding raw pea-flour is indicative that pea, like

Figure 2. Soycomil K, 65% CP, 0.003 mg/gram of CP maximum β-conglycinin. Manufactured by ADM, Elbeweg 139, 3198 LC Europoort-RT, The Netherlands.

soy, is high in antinutritional factors and polysaccharides. Research examining diets formulated with raw pea-flour protein[14,21,51] demonstrates the need to properly process these plant-based proteins. Decreased digestibility of pea-protein-based diets is associated with increased losses of endogenous and bacterial proteins. This loss can occur due to undigested polysaccharides entering the large intestine, where bacteria ferment them similar to ruminal fermentation. Hindgut fermentation, in contrast to ruminal bacterial synthesis where bacteria flow into the abomasum and are digested and absorbed in the small intestine, provides no possibility of bacterial proteins being digested before excretion.[105] In 1 study, the total nitrogen in the digesta from endogenous plus bacterial protein sources doubled during weeks 1 and 4 for the pea-protein diet compared to the skim-milk protein diet.[21] This may be explained partially by the antigenic properties of unrefined pea proteins resulting in sloughing of epithelial tissue into the lumen of the ileum, as well as increased mucin production in response to antigenic processes occurring at the surface of the epithelial mucosa. This is important, because in the young calf most nitrogen absorption (95 to 96%) occurs prior to digesta entering the large intestine.[62] Pea proteins are not routinely used in the US for MR manufacturing.

Similar findings have been associated with poorly processed soybean-based products in calf MR.[63,125,160] Studies discovered sloughing of epithelial tissue in the small intestine, as well as pathological changes in intestinal villus structure and shape, when improperly processed soybean protein (SF,[8,43,99,162,163] SPC[43,162,163]) was incorporated into MR. When allergenic soy proteins were incorporated in MR diets of preruminant calves, intestinal transit time was decreased compared to that of diets formulated with whole milk or caseinate proteins (SF[166,167]). It is hypothesized that decreased transit time allows more time for antigenic proteins to be absorbed and to then stimulate hypersensitivity, with resulting antibody production (raw pea-flour).[21]

Fortunately, soy-based proteins for MR have improved since their introduction and the initial several decades of market presence. Research published in the 1970s and 1980s consistently reported poor digestibility[3] (SF,[4,43] SPC,[4,27,29,43,201] SI[91]), reduced ADG[162] (SF,[43,167] SPC[27,28,29,39,43,201]), and deleterious effects on intestinal morphology (SF,[43,162,163] SPC[43,162,163]) when processed soy was compared to milk-based proteins in calf MR.[168] This contrasts to more current research demonstrating some specifically processed soy performed comparably to milk protein (Soy Milk,[54] SPC[109,181]) when implemented at 25% protein replacement until 49 days of age;[54] at 50% protein replacement until weaned at 2 lb (0.91 kg)/day starter feed consumption;[109] and at 50% protein replacement until 56 days of age.[181] Two more recent research trials examining digestibility of soy products in milk replacers fed to veal calves noted comparable digestibility of SI[98] (but not SF) when replacing 72% of the skim-milk protein and fed to calves either 39 or 95 days of age. Results also showed reduced digestibility when either SF or SPC[33] replaced 50% of the skim-milk protein in the diet of 2 to 3 month-old calves; digestibility of nitrogen was 89%, 89%, and 94% for SF, SPC, and skim-milk protein, respectively. One research trial also noted SI[98] did not produce intestinal pathology. Further research with 9 commercially available processed soy products fed in MR to 2 to 4 month-old calves (Table 2) found apparent digestibility of dietary nitrogen to be highly variable among MR composed of various soy products, with 2 brands of water-extracted and partially proteolyzed soy concentrate having digestibility most similar (86.1% and 87.7%) to skim-milk protein (94.5% and 94.7%). The 2 soy brands processed utilizing this methodology were devoid of anti-nutritional factor β-conglycinin, which these researchers determined was the best predictor of digestibility of soybean N, and produced lower molecular mass proteins when compared to the other processed soy proteins.[99,100] These researchers determined the key relationships among soy product characteristics and apparent digestibility of soybean N were concentrations of β-conglycinin ($P < 0.001$), native protein ($P < 0.01$; i.e. not aggregated-, carbohydrate-linked- or peptide-protein), glycinin ($P < 0.01$), ∞-conglycinin ($P < 0.05$), and lectin ($P = 0.107$). Soy products that most successfully negate these anti-nutritional properties perform adequately.[126] Crude protein level alone may not best describe digestibility of soy-protein sources, because differences in physiochemical properties further affect protein digestibility. For instance, SI, largely produced for food inclusion, may be low or high in solubility dependent on the characteristic needed for specific food applications. Generally, the more insoluble a soy protein is, the more likely it will have a lower digestibility. Soy proteins must be identified and sourced by all of their characteristics and be proven in calf performance trials when formulated in calf MR. Practitioners should request supporting peer-reviewed data from MR manufacturers prior to considering client recommendation. Practitioners can also consider testing soy containing MR for β-conglycinin. A laboratory that conducts this analysis is listed at the end of this paper in the Product and Laboratory List.

Another concern is the sizable carbohydrate fraction present in many commercial soy products. SI is composed of significantly less carbohydrate than SPC, and both SPC and SI

Table 2. Analytical criteria correleated with reduction in apparent digestibility of soy protein in preruminant calves.[99]

Commercial Soy Product (Lallès, et al. *J Dairy Sci* 1996; 79:475†)	Digestibility (%)	β-conglycinin (mg/g of CP)***	∞-conglycinin (mg/g of CP)*	Antitrypsin (TUI3/mg CP)*	Native protein (% of total N)**	Glycinin (mg/g of CP)**
Raw soy flour		155.0	31.5	140.0		269.0
Toasted soy flour 52.9% CP	59	36.1	15.2	19.9	50.9	39.4
Toasted soy flour 52.8% CP	66	13.4	0.45	5.7	17.2	26.8
Toasted soy flour 56.3% CP	76	-	1.13	6.6	15.5	0.7
Water extracted toasted SPC	61	25.5	2.93	6.5	21.6	20.4
Water extracted toasted SPC	71	14.7	3.2	5.2	33.7	32.9
Water extracted toasted SPC	81	-	0.7	2.7	7.9	0.0
Alcohol-extracted heated SPC	81	-	0.45	3.4	9.4	-
Water extracted proteolyzed SPC	82	-	0.0	2.5	2.9	-
Water extracted proteolyzed SPC	84	-	0.4	1.4	4.4	10.5

Simple linear regression between analytical criteria and apparent digestibility of soybean nitrogen
*$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$
†Analysis of results of 4 separate trials in calves 2 to 4 months-of-age
n = 5 to 7 calves/treatment group

contain less carbohydrate fraction than SF. This topic will be further discussed in the carbohydrate section of this review.

Processing raw plant protein sources removes or reduces antigenic and anti-nutritional proteins that are known to diminish calf performance. These antigenic proteins result in physiological damage to the gastrointestinal tract of the calf.[8,92,97] Evaluation of the data indicates that veterinarians should advise clients to restrict the use of soy products in MR to those sources that are: 1) proven void of β-conglycinin and extremely low in other anti-nutritional and potentially antigenic proteins; 2) processed to reduce native protein to very low levels; 3) composed primarily of molecular mass that is less than 20 kilodaltons (kDa); and 4) proven to perform in peer-reviewed calf research studies. Soy-containing products that fail to meet these selection criteria should not be used in MR. In addition, these authors recommend MR containing acceptable soy protein sources be reserved for calves older than 3 weeks. Peer-reviewed and published research examining performance of very young calves fed MR containing soy sources that meet the aforementioned selection criteria should be conducted. Although greater in lysine than SWGP, soy protein is still lower in lysine, methionine, and threonine than milk protein. These synthetic amino acids must fortify soy-containing MR formulas to optimize performance.[86]

Animal Protein Sources

Spray-dried Plasma Protein

Spray-dried bovine plasma (SDBP; Figure 3) is derived from whole blood collected at USDA-APHIS inspected abattoirs. The whole blood is sprayed with calcium citrate at collection, centrifuged to extract red blood cells, and the liquid plasma is spray-dried.

Figure 3. Nutrapro B spray-dried bovine plasma, 78% C.P. Manufactured by APC, 2425 SE Oak Tree Court, Ankeny, IA 50021.

SDBP is a significant source of functional immunoglobulins (IgG), 16 to 16.6% on an air-dried basis.[46,133] While these IgGs are not absorbed into the bloodstream by calves consuming them in a MR diet, in human patients it has been determined that 19 to 20% of the IgGs consumed remain biologically functional throughout passage of the digestive tract.[153]

Inclusion of colostral IgGs in MR diets during the first 14 days of life reduced diarrheal disease in pre-weaned calves raised on calf ranches.[11] Other studies confirm IgG absorbed from colostrum is secreted at the rate of 1 to 4 g/day back into the intestinal tract for the first 14 days of a calf's life.[13,68,157] The IgG fraction of SDBP has been established as the component of SDBP that is responsible for producing enhanced growth rates and improved feed intakes in early weaned pigs.[133] Plasma-based products have been shown to reduce calf morbidity under duress of viral disease challenge.[6]

Researchers in Belgium reviewed studies where bovine colostrum was fed to piglets at or near early weaning. Those authors considered bovine colostrum a natural growth promoter in early weaned piglets, and attributed the growth promotion to multiple factors providing synergistic effects to preserve intestinal integrity.[20] Feeding bovine colostrum increased daily feed intake, maintained higher villi structure, reduced crypt depth, and produced an increase in duodenal protein synthesis.[104] Two studies document the impact of orally administered bovine IgGs on intestinal health. During enterotoxigenic *Escherichia coli* challenge, intestinal health and performance parameters were improved.[93,129] Authors in 1 of the studies tested the impact of 3 antibody sources fed to 10-day-old piglets challenged with enterotoxigenic *E. coli*: porcine plasma, animal plasma that was mainly bovine plasma, and egg antibodies. Although the porcine plasma and animal plasma sources had different antibody concentrations to different *E. coli* strains, the outcomes of feeding both plasma sources were not different. Both sources were identified as important therapeutic and prophylactic baby pig feed nutrients.[129] Adding strain-specific IgY antibodies only marginally improved early-weaned pig performance. In the presence of human immunodeficiency virus-induced enteropathy, daily consumption of 5 grams of bovine serum-derived IgG increased intestinal mucosal CD_4^+ lymphocyte counts, improved duodenal function, and promoted intestinal repair in human AIDS patients.[7]

There are 8 published papers encompassing 9 controlled calf trials comparing spray-dried animal plasma to milk-based formulas (1 trial compared plasma to soy and a second, where ADG was not measured, compared serum to soy) (Table 3).

In all 7 trials where ADG was measured, calves fed plasma protein performed as well as those fed milk-based formulas;[6,138,142,143] in 2 trials plasma-based formulas outperformed milk-based formulas ($P < 0.05$);[119,139] and in 1 trial, plasma tended ($P < 0.1$) to outperform[142] the all-milk formula. Plasma inclusion rates, where ADG was measured, included

3.5%,[139] 4%,[142] 2 trials at 5%,[142,143] 7%,[119] 7.5%,[138] and 1 trial that examined a 160 g/day dose.[6]

Four of the 8 studies were disease-challenge trials. A 75 g/day dose of plasma (either beef or pork) reduced mortality and pathogen shedding ($P < 0.05$) when colostrum-deprived calves were challenged with virulent strains of *E. coli* (100% mortality in the control group).[124] Another trial compared either a modest 3.3% inclusion rate of plasma or 800:400 levels of neomycin:oxytetracycline to an all-milk, non-medicated control formula.[139] This trial revealed that feeding plasma improved attitude and hydration scores and ADG ($P < 0.05$), while tending ($P < 0.06$) to reduce mortality. Calves fed MR formulated with plasma performed similar to calves in the antibiotic treatment group.

Two of the 8 trials were conducted using spray-dried serum, which is plasma minus fibrinogen. One trial compared 2 oz (56.7 g) daily intake of either bovine serum or soy protein concentrate fed in addition to a whey-based MR to calves orally inoculated with *Cryptosporidium parvum* oocysts.[78] Supplementation of serum reduced oocysts shed and peak scour volume ($P < 0.05$), and a chromium EDTA assay method also indicated a reduction in gut permeability ($P < 0.05$) with serum supplementation. Calves were sacrificed at day 18 and it was determined that supplementation of serum improved crypt depth and villous surface area ($P < 0.05$). The second serum trial examined feeding high rates (5.6 oz (158.8 g) daily) of a predominantly serum-containing product (Lifeline™) to calves orally challenged with a virulent strain of bovine coronavirus. Feed intake and level of hydration improved ($P < 0.05$), and there were trends toward reduced respiration rates ($P < 0.06$).[6]

Among these 9 trials, 3 reported improvements ($P < 0.05$) in starter grain intake,[6,119,143] and 3 also reported less ($P < 0.05$) mortality[124,142,143] due to feeding plasma proteins. Otherwise, calves supplemented with plasma in all other trials had comparable starter intake and mortality with those fed control formulas.

Typical plasma inclusion rate is 5% (100 lb (45.4 kg)/ton of MR powder), replacing approximately 20% of the milk-based protein in the formula. Plasma has an excellent amino acid profile that is comparable to milk protein. Small quantities of DL-methionine and lysine bridge minor gaps in these amino acids compared to milk. Blood-based proteins are lower in isoleucine relative to milk protein. Very high health-status calves, with 4% and 8% inclusion rates of plasma in MR, performed similarly to an all-milk protein-based formula. When synthetic isoleucine and threonine were added to the 8% plasma formula to match those in milk protein, starter intake increased, exceeding the all-milk ($P < 0.01$), but no improvements in ADG were noted.[70] Adding synthetic threonine and isoleucine tended ($P = 0.08$) to improve ADG in a 10% plasma formula fed at an average of 1.72 lb (0.78 kg)/day for 35 days.[120]

In addition to the aforementioned peer-reviewed published research, there are also 11 abstracts examining spray-dried plasma proteins in MR published in the *Journal of Dairy Science*. Of the 10 that reported ADG, calves in 6 studies performed comparably to those fed an all-milk formula,[30,70,181,195,196,203] calves in 1 study out-performed ($P = 0.05$) those fed the all-milk product at 14 days of age,[134] and another report noted improved ($P < 0.05$) performance in 53-day-old veal calves when plasma feeding rates were increased.[198] One trial noted poorer ($P = 0.05$) performance compared to the all-milk MR during the post-spray dried-serum feeding period (23 to 60 days of age),[108] perhaps indicative of difficulties withdrawing supplementation of spray-dried serum at 24 days of age on a commercial calf ranch. A second is the aforementioned trial where there was a trend ($P = 0.08$) toward poorer performance when 10% SDBP MR was not supplemented with threonine and isoleucine. The other 3 plasma treatments in this trial performed comparably to the all-milk MR.[120] One trial noted increased ($P = 0.03$) 28-day plasma total protein,[135] while a second trial noted increased ($P = 0.03$) serum total protein on days 8, 15, and 24 when spray-dried serum was fed.[108] In 4 trials, a combination of plasma and SWGP performed comparably to an all-milk MR,[30,181,196,203] and in 2 trials a combination of plasma and SPC performed comparably to an all-milk MR.[181,203]

Table 3. Published‡ results of feeding plasma products to milk-fed calves

Reference	Diet CP & fat, %	% plasma (beef or pork); % of absolute formula, or % of CP, if designated	ADG, lb/d	Scour score	Death %	Starter intake lb/day	Age of calves, d
Morrison S. *J Dairy Sci* 2014; 97 (Abstract)[120]	22% CP	All-milk (AM)	1.054	†	-	0	1 - 35
104 calves (20 or 21/group)	20% fat	5% beef + methionine	1.111	-	-	0	1 - 35
10% of BW d 1 - 2, 12% d 3 - 7, 14% d 8 - 35,	2% lysine	5% beef + a.a. meth, Ile & Thr††	1.019	-	-	0	1 - 35
2x/d		10% beef + methionine	0.902*	-	-	0	1 - 35
Avg daily MR intake: 1.72 - 1.78 lb		10% beef + meth, Ile & Thr††	1.069	-	-	0	1 - 35
No grain							
Ziegler D. *J Dairy Sci* 2014; 97 (Abstract)[203]	20% CP	All-milk	No diff ($P = 0.11$) in 56 day ADG. Calves doubled initial BW				
105 (26 or 27/group)	20% fat	50% of CP wheat/plasma combo.	Wheat/plasma improved ($P < 0.05$) pre-weaning ADG vs				
48.1 lb MR/calf		50% soy protein con.(SPC)/	Fecal scores higher ($P < 0.05$) for AM vs all other treatment groups				
Calf starter (CS) 18% CP, free choice		plasma	SPC/plasma or wheat/plasma/SPC				
		50% of CP wheat/plasma/soy					

Wood D. *J Dairy Sci* 2014; 97 (Abstract)[198]	#	Basal ^ diet + 2.5 lb added WPC	1.712	▫ 30%	0%	0	1 - 53
(all-milk 44, plasma 42, colostrum 42 head)		Basal + 2.5 lb added plasma ^	1.817**	▫ 21%	0%	0	1 - 53
126.8 lb MR/calf, no grain. veal		Basal + 2.5 lb added colostrum ^	1.763	▫ 17%	0%	0	1 - 53
Wood D. *J Dairy Sci* 2013; 96 (Abstract)[197]	##	All-milk	1.912	▫ 42%	13.2%	0	1 - 56
(all-milk 41, plasma 41, colostrum 38 head)		6.7 lb beef plasma/calf ^^	1.886	▫ 19.5%**	4.8%	0	1 - 56
148 lb MR / calf, no grain. veal		11 lb colostrum/calf ^^	1.92	▫ 17%**	4.8%	0	1 - 56
Pineda A. *J Dairy Sci* 2011; 94 (Abstract)[135]	20% CP	Electrolyte w/serum + 14 d	Gammulin resulted in greater 14 d (*P* = 0.05) BW				
Pinead A. *J Dairy Sci* 2010; 94 (Abstract)[134]	20% fat	Gamm	Gammulin resulted in lesser (*P* = 0.007) 56 d mortality				
93 calves; groups stratified by BW & plasma	1 arrival	Regular electrolyte + 14 d Gamm	Acute phase response was not affected by any treatment				
protein	meal of elect.	Electrolyte w/serum, no	Gammulin resulted in greater (*P* = 0.04) 28 d plasma total protein				
Gammulin (Gamm) = serum + FOS; 0.11/calf/		Gammulin					
day		Regular electrolyte, no Gammulin					
Wood D. *J Dairy Sci* 2009; 92 (Abstract)[196]	20% CP	All-milk	1.41	-	10%	-	1 - 42
(60 hd/diet); 57.9 lb MR/calf; free choice CS,	20% fat	5% beef & 6% wheat	1.44	-	7%	-	1 - 42
17% CP							
Lopes G. *J Dairy Sci* 2009; 92 (Abstract)[108]	whole milk	Pasteurized waste milk	Control calves gained more (*P* = 0.05) d 23-60 (1.27 vs 1.2 lb/d)				
255 head control; 263 head Gammulin (d	MR d 23-60	Past. waste milk + 0.055 lb/d	Gammulin increased (*P* = 0.03) serum total protein day 8, 15 & 24				
1 - 24)		Gamm					
Quigley JD. *J Dairy Sci* 2006; 89:207[144]	20:20 CP:Fat	All-milk MR 28 day wean	1.03	1.44	8.6%	2.02	1 - 56
(40 hd/diet) Gammulin (serum) 1.5 lb over	28:16 CP:Fat	All-milk MR 42 day wean	1.32****	1.6***	22.3%	1.64***	1 - 56
15 days	28:16 CP:Fat	+Gammulin 42 day wean	1.28****	1.56***	12.6%	1.53***	1 - 56
20:20 28 lb MR/calf, 28:16 60.5 lb MR/calf							
Jones C. *J Dairy Sci* 2004; 87:1806[85]	22% CP	All-milk	Primarily a colostrum replacer study. Data not reported.				
40 calves all milk, 38 calves plasma	21% fat	5.8% animal plasma	Summary: "Calves fed animal plasma gained at the same rate"				
Hayes S. *J Dairy Sci* 2009; 92. (Abstract)[70]	20% CP	All-milk	1.21	1.27	0%	0.91	1 - 42
123 calves (grouped 32, 31, 31 & 29)	20% fat	4% plasma	1.21	1.26	0%	0.87	1 - 42
48.2 lb MR offered/calf		8% plasma	1.21	1.24	0%	0.87	1 - 42
Free choice CS 21.7% CP		8% plasma + threonine &	1.30	1.25	0%	1.119**	1 - 42
		isoleucine					
Carlson D. *J Dairy Sci* 2009; 92 (Abstract)[30]	20% CP	All-milk	1.26	1.54**	0%	1.41**	1 - 56
102 calves (25 or 26/group); free choice CS	20% fat	All-milk, step-down to 1 lb/d,	1.32	1.46	0%	1.74	1 - 56
All-milk 1.25#/calf/d, 48.3 lb MR/calf	wean d 42	d 15	1.30	1.44	0%	1.65	1 - 56
All-milk, plasma & wheat plasma step-down,		Plasma, step down to 1 lb/d, d 15	1.19	1.44	0%	1.61	1 - 56
41.9 lb MR/calf		Wheat/plasma, step down too					
Quigley JD. *J Dairy Sci* 2003; 86:586[143]	20% CP	All-milk	0.51	1.67	25%	1.457	1 - 42
(40 hd/diet)	20% fat	5% beef plasma	0.575	1.58*	7.5%**	1.565*	1 - 42
49.3 lb MR/calf; free choice CS, 21.6% CP		5% pork plasma	0.48	1.61	5%**	1.508	1 - 42
Quigley JD. *J Dairy Sci* 2002; 85:413[142]	22% CP	All-milk + placebo	0.93	1.60	3.3%	1.085	1 - 56
(30 hd/diet)	20% fat	All milk + Gammulin	1.01*	1.60	0%	1.133	1 - 56
49.3 lb MR/calf		5% Beef + placebo	0.95	1.60	0%	1.107	1 - 56
2 x 2 latin square with plasma &		5% Beef + Gammulin	1.02*	1.60	0%	1.179	1 - 56
Gammulin (bovine serum + FOS + vitamins)	22% CP	13% soy protein concentrate (SPC)	1.02	1.49	20%	1.29	1 - 56
Gammulin fed 1.5 lb/calf over 15 d	20% fat	13% SPC + Gammulin	1.09	1.44*	3.3%**	1.30	1 - 56
Placebo was isonutritious to Gammulin	13% SPC	13% SPC, 4% beef plasma	1.09	1.47	3.3%**	1.29	1 - 56
CS available free choice day 29 onward		13% SPC, 4% beef + Gammulin	1.17	1.45*	6.7%**	1.37	1 - 56
Catherman D. *J Dairy Sci* 2001; 84 (Abstract)[31]	22% CP	All-milk, 1 lb MR/calf/day	1.18	no	-	1.96	1 - 42
85 calves (21 or 22/group); Weaned at 35 days	20% fat	5% plasma, 1 lb MR/calf/day	1.24	difference	-	2.01	1 - 42
29.8 lb or 39.7 MR/calf, 1 & 1.5 lb/d,		All-milk, 1.5 lb MR/calf/day	1.26	in scour	-	1.80	1 - 42
respectively		5% plasma, 1.5 lb MR/calf/day	1.24	score	-	1.89	1 - 42
CS available free choice (18% CP)							
Quigley JD. *Food Agr Immunol* 2002; 12:311[139]	20% CP	All-milk	1.09	0.62	25%	-	1 - 21
(12 hd/diet) *E coli* challenge 108 Day 3	20% fat	Neo-Terra 800:400	1.39**	0.72	8.3%	-	1 - 21
1 lb MR/calf/day; CS free choice d 7 - 21		3.3% Beef	1.46**	0.74	0%	-	1 - 21
Arthington JD. *J Dairy Sci* 2002; 85:124[6]		1 lb 20:20 MR	1.59	1.61	0%	ǂ 0.97	1 - 14
Coronavirus challenge (12 hd total); CS free		0.7 lb Lifeline (serum)	1.47	1.73	0%	ǂ 1.25**	1 - 14
choice							
Nollet H. *Am J Vet Med* 1999; 46:185[124]	Sterilized	Milk only	-	2.00	100%	-	1 - 7
E coli 105 cfu challenge at 12 - 24 hours age	whole milk	+75 g/day beef plasma	-	0.60	0%**	-	1 - 38
6 hd/diet; Colostrum deprived calves. CS d 7	2 liters,	+30 g/day beef plasma	-	0.70	17%**	-	1 - 38
Plasma pasteurized 122 °F for 15 m prior	3x daily	+75 g/day pork plasma	-	1.00	50%**	-	1 - 38
drying							
Quigley JD. *J Dairy Sci* 1996; 79:1881[138]	20% CP	All-milk	1.15	1.37	0%	1.34	1 - 56
(33 hd plasma, 35 all-milk) MR 10% of BW;	20% fat	7.5% beef plasma	1.03	1.40	6%	1.20	1 - 56
CS ad lib							

Morrill JL. *J Dairy Sci* 1995; 78:902[119]	20% CP	All-milk + probiotic	0.53	1.80	0%	1.30	1 - 42
Ramped up to 1 lb/d MR	20% fat	All-milk + CTC	0.56	1.80	6.6%	1.281	1 - 42
Weaned when 1.5 lb/d starter intake		7% pork + CTC	0.7**	1.80	3%	1.61**	1 - 42
(30 hd/diet) Chris Hansen Probiotic		7% beef + CTC	0.66**	1.80	10%	1.40**	1 - 42
Tomkins T. *J Dairy Sci* 1994; 77 (Abstract)[181]	-	(A) All-milk	0.882	-	0.0%	1.398	1 - 56
(240 calves randomly assigned 8 groups)	-	(B) 50% SPC	0.960	-	6.7%	1.61	1 - 56
Mortality column are morbidity/removed		(C) 50% SPC, 10% SWGP	0.871	-	6.7%	1.446	1 - 56
50 lb MR/calf; CP & fat not reported		(D) 50% SPC, 20% SWGP	0.780	-	13.3%	1.387	1 - 56
starter grain intake day 15 - 56		(E) 40% SPC, 10% SWGP	0.800	-	3.3%	1.382	1 - 56
Abstract reports no difference in health		(F) 40% SPC, 20% SWGP	1.070	-	10.0%	1.882	1 - 56
Day 1 - 14 ADG was superior ($P < 0.05$) for		(G) 50% SPC, 10% Plasma	0.860	-	10.0%	1.516	1 - 56
all-milk		(H) 50% SPC, 10% SWGP, 10%	0.811	-	20.0%	1.403	1 - 56
vs treatments C, D or H		Plasma					

*$P < 0.10$, **$P < 0.05$, ***$P = 0.02$, ****$P < 0.001$

†21 d scour occurance odds ratio was 1.35 ($P = 0.07$) & 1.61 ($P = 0.01$) greater for AM vs 10% plasma & 10% plasma + a.a (amino acids), respectively; 5% plasma+ a.a. tended ($P = 0.08$) greater scour occurance odds ratio (1.41) than 10% plasma + a.a.

Basal diet contained 5.2% beef plasma. Calves started on 0.75 lb 25% CP: 18% fat MR; eased up to 1.92 lb 21:18.2 day 21 and 3.84 lb 20:18 at day 53; ## calves started on 0.63 lb 25.8% CP:19.89% Fat; eased up to 2.94 lb 20:19 day 28 and 4.2 lb 18:20 by day 56; calves treated week one, period of peak scours

▫ denotes calves treated week of peak scours

^ Colostrum/plasma standardized for IgG, isonutritious as possible. Colostrum 13% IgG 44% CP, 18% fat; plasma/fat mix 13% IgG, 70% plasma, 57% CP, 18% fat WPC/fat blend 44% CP, 18% fat

^^ Isonutritious formulas, Colostrum 50% CP: 20% fat; 60% plasma, 40% dry fat blend or WPC/dry fat blend, both equaling 50% CP:20% fat, added to feeding bowls

ǂ Total feed intake (MR and starter grain combined)

††Synthetic methionine, isoleucine & threonine; a.a. means amino acids

Spray-dried Egg Protein and assorted other Protein Sources

Various forms of egg protein have been evaluated in MR formulas, at increasing inclusion rates. Egg protein sources invariably include egg-white protein, which contains the anti-biotin agent avidin;[102,132] ovomucoid, a protinease inhibitor;[87] and ovoinhibitor, a trypsin and chemotrypsin inhibitor.[87] There is an upper limit for inclusion of egg proteins, beyond which a detrimental impact on performance is observed[141,161,183] (Table 4). Two studies demonstrated equal or improved ADG and starter feed intake with specific sources of egg protein in MR.[71,88] Review of egg protein in MR studies is difficult, as some researchers added feed-grade liquid egg to MR and some added feed-grade spray-dried egg to MR dry formulations. Adequate pasteurization procedures would be essential for either source of egg protein. Potential contamination with *Salmonella* spp is possible.[52]

Other novel sources of animal protein have been evaluated for inclusion into calf MR, including spray-dried red blood cells, spray-dried fish solubles, and hydrolyzed animal proteins, but to our knowledge none of these proteins are routinely used by manufacturers in North American or European MR markets, and are not considered further in this review.[27,28,46,63,77,140,177]

Amino Acid Profiles

When alternative proteins are formulated into calf MR, it is necessary to consider amino acid (AA) supplementation to balance the overall AA profile of the MR powder fed. Tables 5 and 6 compare essential AA profiles of several alternative protein sources to that of whole milk, calf muscle tissue, and various whey protein concentrates.

Effects of Enzymes and Colostrum Management on Digestive Physiology

Veal-calf studies determined enzyme activity in preruminant calves that were fed either skim-milk powder, non-hydrolyzed or partially hydrolyzed soy-protein concentrate, or potato-concentrate formulated calf MR.[113] The MR diets were continuously infused into the abomasum. Mucosal biopsies and digesta from the duodenum, jejunum, and ileum were evaluated. Chemical activity of dipeptidylpeptidase IV, aminopeptidases, lactase, and alkaline phosphatase were estimated in both biopsy tissue and digesta collected. Potato protein had limiting impact on intestinal mucosa, but both soy-protein concentrates produced partial villus atrophy and crypt hyperplasia. Dipeptidylpeptidase IV activity was reduced by potato concentrate. The partially hydrolyzed soy concentrate produced lower aminopeptidase N and lactase activity. More enzymes of all types were discovered in the distal ileal digesta for plant protein diets, compared to digesta evaluated from calves consuming diets formulated with skim-milk powder.[113] These data suggest that further refinement of plant proteins is necessary in order to appropriately incorporate them into a calf MR, especially for calves early in their MR feeding period.

Adequate colostrum intake has been defined as 4 quarts (3.79 L) of colostrum containing at least 50 grams/quart (0.95 L) of IgG immediately following birth.[36,179] Postnatal

Table 4. Published results of feeding egg products to preweaned calves.

Reference	Diet CP and fat, %	% egg	ADG, lb/d	Feed efficiency (gain:feed)	Scour score	Death, %	Age of calves, d
Quigley JD. *J Dairy Sci* 2002; 85:198-203* (40 hd/diet)[141]	22% CP 20% fat	0 10 20	0.51‖ 0.15 (-70%) 0.00 (-100%)				6-34
		0 10 20	1.07§ 0.81 (-24%) 0.67 (-38%)	0.45‖ 0.32 (-29%) 0.23 (-48%)	1.61§ 1.72 1.67	0 6.7 0	6-62
Scott TA, et al. *J Dairy Sci* 1999; 82 (Suppl. 1):46(abstr) (43 hd/diet)[161]	20% CP 20% fat	0 8.5 17	0.29‡ 0.02 (-92%) -0.13 (-145%)				1-14
		0 8.5 17	0.70§ 0.48 (-31%) 0.42 (-40%)	0.67‡ 0.63 (-6%) 0.60 (-10%)	1.76 1.87 1.87	4.6‡ 23.3 9.3	1-56
Hill TM, et al. *J Dairy Sci* 2001; 84 (Suppl. 1):265 (abstr) (10 hd/diet)[71]	20% CP 20% fat	0 3.4 6.8 10.2	No actual numbers published, but egg content at 6.8% or higher tended ($P < 0.1$) to reduce gain				Starting wt 90-95 lb
Touchette KJ, et al. *J Dairy Sci* 2003; 86:2622-2628† (20 hd/diet)[183]	20% CP 20% fat	0 5	0.71‡ 0.81 (14%)	No difference			Starting wt 95-99 lb
		0 5	1.07 1.13	No difference			Starting wt 90-95 lb
		0 5 10 15	1.39 1.39 1.41 1.17	No difference			Starting wt 90-95 lb
Kellogg DW, et al. *AAES Res Ser* 2000; 478:149-153 (8 hd/diet)[88]	20% CP 20% fat 26% CP 15% fat	0 30	0.49 0.48	Starter intake severely restricted (<15 lb total for 4 weeks) but control calves consumed slightly more			4-7 days, on feed 28 d

*Added biotin had no effect.
†Weaning and weighing ages were variable between treatments, and may have contributed to the reported differences.
‡$P < 0.05$
§$P < 0.01$
‖$P < 0.001$

Table 5. Amino acid profile comparison. Typical analysis: grams amino acid/100 grams of dry protein presented on a 100% protein basis.

	Dried skim milk‡	Cow's milk*	Calf muscle tissue†	MSC WPC 34%	Proliant 8000 WPC§ 80%	APC NUTRAPRO Plasma	MGP hydrolyzed SWGP	Soy Protein Conc. HP100
Lysine	8.71	11.70	8.40	9.55	13.12	9.37	1.54	6.53
Methionine	2.98	3.60	N/A	1.79	2.55	0.96	1.41	1.39
Threonine	2.51	6.40	4.30	7.17	8.27	6.61	2.74	4.17
Isoleucine	5.99	8.20	5.20	6.06	7.51	4.00	2.76	4.92
Leucine	9.31	13.60	7.30	10.63	12.99	0.11	6.32	8.24
Tryptophan	2.03	2.00	1.30	2.09	2.17	1.93	0.62	1.44
Histidine	3.33	3.80	3.20	1.94	1.91	3.86	2.03	2.78
Valine	5.49	9.20	5.10	5.91	7.13	7.31	3.27	5.13
Arginine	4.37	5.00	6.50	2.56	2.93	6.50	3.12	7.70
Glycine	2.82	N/A	N/A	2.03	2.04	4.14	3.60	4.60
Aspartic acid	8.42	N/A	N/A	11.34	13.25	10.90	3.16	11.90
Glutamic acid/glutamine	23.88	N/A	N/A	18.54	21.40	16.13	35.63	19.36
Cystine	N/A	1.10	N/A	2.51	3.57	3.86	1.77	1.50
Phenylalanine	5.52	6.80	N/A	3.28	3.82	6.34	5.10	5.35
Tyrosine	7.36	7.10	N/A	2.63	4.33	4.96	2.86	3.96
Meth + cyst	N/A	4.70	3.50	4.30	6.12	4.82	3.18	2.89

*From Table 14.2, Amino Acid Requirements of the Veal Calf and Beef Steer. AP Williams[193]
†Gordon et al, 1965[58]
‡Van Weerden and Huisman text reference[187]
§WPC is whey protein concentrate, 80% CP.

colostrum provision is necessary for proper development and maturation of intestinal mucosa.[96,130,158,199] The 4 quart (3.79 L) colostrum feeding recommendation is based on the assessment of 919 first-milking colostrums by Washington State University researchers.[53,137] They reported that 87% of the 919 colostrums would have provided 100 grams of IgG, and there was a correlation between weight of colostrum at first milking and IgG content, but did not measure accurately against time of first milking as all samples were taken from colostrums milked within 8 hours of calving. More recent research determined that a strong correlation exists between IgG content and time of first milking, and led to a recommendation to harvest colostrum within 2 hours of calving.[118] University of Missouri researchers recommended colostrum IgG content be assessed with a BRIX electronic, temperature-compensated refractometer,[36] and recommended calves be fed 4 quarts (3.79 L) of colostrum with at least 50 grams of IgG/quart (0.95 L) (22-23 BRIX). No correlation was noted between weight of colostrum at first milking and IgG content, R^2 value=0.03. Colostrum contains 90 to 95% IgG_1,[101] insulin-like growth factor-1, transforming growth factor beta-2, and growth hormone.[49] It has been determined that transforming growth factor beta-2 inhibits intestinal cell proliferation in cell culture, but stimulates cell differentiation.[96]

Measurements taken at 5 days of age indicated that colostrum administration increased villus size in the jejunum and ileum, enhanced xylose absorption, and increased peptidase activities in the ileum. Intestinal responses to dexamethasone injections, utilized to stimulate mucosa and organ maturation in immature or premature calves,[159] were accentuated in calves administered colostrum. An earlier study demonstrated colostrum intake did not positively impact xylose absorption,[149] but in this study colostrum quality (IgG content) was not assessed. Colostrum was fed as a nutrient in varying quantities through 7 days of age. In a 2012 review of published research, German authors concluded postnatal glucose metabolism is dictated by perinatal maturation of endogenous glucose production, and proper colostrum provision improves glucose absorption and liver storage of glucose. Both processes make glucose more available to peripheral tissues.[65]

To maximize growth and development in neonatal calves, a high quality, digestible protein is necessary.[77] Numerous research studies have confirmed that alternative non-dairy proteins are digested more efficiently as calves progress in age.[3,27,28,33,73,99,123,147,162,190,191,201] It has been postulated that reduced digestibility, resulting in impaired performance parameters when alternative plant proteins are used in calf MR, particularly when SPC is used, is due to lack of specific digestive enzyme secretion.[3] As has been reviewed previously, some alternative plant origin proteins — soy and potato specifically — also produce pathological tissue changes in the intestinal tract, presumably due to their antigenic reactivity. It has also been postulated that improved digestibility of alternative plant-sourced proteins in older calves can be explained by maturation of digestive tract physiology that occurs over time, as well as increasing enzyme production and activity. Digestive enzyme activity does increase with age, and the quantity of enzyme recovered from the ileum digesta indicates that more total enzyme is secreted in response to the presence of plant proteins in the diet.[113]

Carbohydrates

An extensive research study of carbohydrate digestive enzyme activity in the bovine was published in 1968.[164] The study determined that neither the calf nor the adult cow secretes sucrase. The data agreed with earlier studies in sheep, where no intestinal sucrase activity was identified.[110,188,189] Sucrose is used as a feed source for cattle of all ages, but its use in neonatal calves should be limited due to the osmotic pull of undigested sucrose within the lumen of the intestinal tract. Earlier work did postulate small quantities of sucrose are digested within the distal portions of the digestive tract by microorganism flora.[45,75,76] As the calf matures and develops a functional rumen, enzymes secreted by rumen flora digest sucrose in dry feed, such as the sucrose in molasses added to dry feed as a texturizer.

Intestinal amylase and dextranase were also found in homogenates of intestinal mucosa in limited quantities, but their activity was distributed along the entire small intestine. Amylase is an enzyme that hydrolyzes starch, a conglomerate polymer of glucose, to individual glucose or dextrose molecules, while "dextrose" is the commercial nomenclature for glucose. Dextrin, a short-chain polymer of glucose, is composed of 3 glucose residues, 2 of which are in the chemical structure of an isomaltose.

Homogenates of pancreas demonstrated only 2 carbohydrase enzymes, amylase and maltase. High amylase activity was discovered in pancreatic secretions, which increased with age. Again, maltase activity was very low, and did not increase with age. Calves fed fructose demonstrated little fructose uptake, and severe diarrhea resulted from its feeding.[188] Calves fed inverted sucrose (sucrose treated with invertase (sucrase) for 10 minutes pre-feeding) did not experience diarrhea, and demonstrated good sugar uptake from the intestinal tract. This may be due to the change in isomer optical angles produced by sucrase enzyme activity, which inverts the geometric shape of both glucose and fructose from –39.5 degrees with some β-D isomers and L-isomers to +66.5 degrees and 100% α-D isomers of glucose and fructose. Feeding inverted sucrose produced no diarrhea, and uptake of sugars into the bloodstream was comparable to oral glucose solution feeding. The utilization of corn syrup[a] was tested on 4, 8, and 11-week-old calves. While blood sugar did rise moderately, calves administered corn syrup scoured frequently within 4 hours of feeding. Raw corn syrup is 30 to 70% dextrose (glucose) with 8 to 45% mono-, di-, tri-, tetra-, penta-, hexa-, and hepta-saccharides, depending on whether or not the corn syrup has been acid, enzyme, or

Table 6. Amino acid profile comparison (%). All examples are as-is; all comparisons are gram/100 grams (as-is) from manufacturer specification sheets*.

	Proliant 8000 WPC 80%	MGP hydrolyzed SWGP	NUTRAPRO spray-dried animal plasma	Soy protein concentrate (Hamlet HP100)	MSGAN whey protein conc 34%
% Protein	82.00	87.00	78.00	57.50	34.00
Dry matter	95.40	96 – 98	93.00	93.50	98.46
Lysine	10.30	1.30	6.80	3.51	3.20
Methionine	2.00	1.19	0.70	0.75	0.60
Threonine	6.50	2.31	4.80	0.22	2.40
Isoleucine	5.90	2.33	2.90	2.65	2.03
Leucine	10.20	5.33	7.80	4.43	3.56
Tryptophan	1.70	0.52	1.40	0.78	2.40
Histidine	1.50	1.71	2.80	1.50	0.65
Valine	5.60	2.76	5.30	2.76	1.98
Arginine	2.30	2.63	4.70	4.14	0.86
Glycine	1.60	3.04	3.00	2.47	0.68
Aspartic acid/asparagine	10.40	2.67	7.90	6.38	3.80
Glutamic acid/glutamine†	16.80	30.07	11.70	10.41	6.21
Cystine	2.80	1.50	2.80	0.81	0.84
Phenylalanine	3.00	4.31	4.60	2.88	1.10
Tyrosine	3.40	2.41	3.60	2.13	0.88
Proline	5.90	13.23	4.50	2.93	2.17
Serine	5.10	4.16	4.70	2.99	1.92
Meth + cystine	4.80	2.69	3.50	1.55	1.44
Crude Protein	80.00	87.00	78.00	57.50	34.00
Crude Fat	4.30	4.73	0.30	2.50	3.00
Starch or lactose	4.30	7.60	0.00	30.00	52.10
Ash	2.80	1.27	8.50	n/a	5.95
Dry matter	95.40	95.50	93.00	93.50	98.46

*Product manufacturers identified in Product List shown elsewhere in this paper.

†Hydrolyzed wheat protein is primarily glutamine, not glutamic acid.

acid-enzyme treated. Processing with isomerases converts a portion of the dextrose to fructose. The dry matter content of the isomerase-treated corn syrup is 50% dextrose, 42% fructose, and 8% other saccharides. This isomerase-treated corn syrup is marketed as high fructose corn syrup and is used as a commercial sweetener, comparable in perceived sweetness to that of sucrose.[50] A commercial blend of corn syrup-high fructose corn syrup was used in this research.[a]

In an 8-week study, male and female calves were fed MR where a portion of the lactose content was replaced by corn-syrup solids: 1) control diet 33% lactose whey; 2) 25% lactose whey + 20% corn-syrup solids; 3) 33% lactose whey + 10% corn-syrup solids; and 4) 25% lactose whey + 10% corn-syrup solids + 10% dextrose.[12] Calves were fed 1 lb (0.45 kg) MR powder/day for 5 weeks, then 50% feeding daily for 1 week, and then weaned at the end of the sixth week. Average scour score was higher for treatments 2 and 4 ($P = 0.02$) compared to treatment 1. Weight gains, starter intakes, heart girths, and body length measurements were not different among diets. The authors stated corn-syrup solids could be utilized to replace a portion of lactose whey in milk replacers, but there is no advantage to feeding a combination of lactose whey, corn-syrup solids, and dextrose. Economics would dictate the demand for corn-syrup solids as an alternative carbohydrate source for calf milk replacers. It is unknown if the corn-syrup solids contained significant fructose.

Glycerol (glycerin) is industrially produced by strong base hydrolysis of fat sources, which is irreversible, yielding glycerol and free fatty acids.[40] Glycerol is a 3-carbon alcohol entering the Krebs Cycle at the same point as citric acid, each molecule of glycerol potentially providing one-half the energy of a molecule of glucose. Its physical form is a viscous liquid. Most commercially available glycerol is the by-product of biodiesel manufacturing. It is contaminated with varying amounts of methanol and sodium hydroxide, which temper its use. Glycerol often is uneconomical as an energy source in MR or feed, due to its significant commercial and industrial demand.

Glycerol was added to premixed MR at 15% total dry matter in replacement for lactose.[48] It replaced up to 37.5% of total lactose and produced no negative consequences for calves consuming it. Growth and health parameters were not significantly different from those for calves consuming a control diet of commercial MR without glycerol. One study identified the impact of glycerol addition to dry grain mixes for Holstein heifer calves, reporting no negative consequences to health and performance.[55] Another study determined that glycerol could replace approximately 46% of lactose in calf MR without negatively impacting calf growth or health.[148]

Little or no maltose utilization was observed in calves fed 2 grams of maltose/lb (0.45 kg) of body weight, following a 24-hour fast.[164] Feeding a warm water solution of maltose produced severe diarrhea within 3 to 4 hours of feeding, and continued for 5 hours after feeding in calves of various ages. This was as expected, since maltase activity in calves was determined to be limited for the entire length of the digestive tract. Corn syrup contains small amounts of maltose, along with other more complex sugars.

Aminopeptidases A and N, alkaline phosphatase, lactase, and isomaltose were evaluated from 2 days through 119 days of age in milk-fed calves.[103] Enzyme levels were highest at day 2, declined between days 2 and 7, but remained largely unchanged thereafter. Maltase activity increased from day 7 to 119, although in very limited quantities, but no sucrase activity was identified at any age. Weaning calves from milk resulted in a decline in activity of lactase and an increase in activity of aminopeptidase N, maltase, and isomaltose.

Disaccharidase, α-amylase, lipase, proteinase, and trypsin enzyme activity were elevated in calves receiving more feed and gaining more weight, but enzymatic activities of pancreas and small intestinal mucosa were not affected by the plane of nutrition.[191] Authors postulated as calves develop and grow in weight, the weight of enzyme-producing tissues increases, primarily due to the presence of more enzyme-producing tissue in larger, growthier calves fed more total feed. Heavier calves on a high plane of nutrition had greater ability to digest starch. These authors determined there is adaptation by the digestive system to increase digestive capacity as food source quantity increases. Others have documented this adaptive capability in calves simultaneously infected with rotavirus and enterotoxigenic *E. coli*.[175] These data agreed with that collected from calves where intestinal length was diminished by surgical resection.[67] These studies confirm that the intestinal mucosa and the pancreas adapt to loss of absorption capacity by increasing enzyme production.

An extensive literature review confirmed the calf has an adaptable digestive system.[61] Development of the gastrointestinal tract depends on gut regulatory peptides, plasma concentrations of metabolites, and gut luminal concentrations of nutrients. While some intestinal enzymes are present at birth – namely chymosin, elastase II, and lactase – pepsin, ribonuclease, and amylase increase in quantity with time in order to support the neonatal calf at the time of weaning. One study indicated this adaptability can be overcome with nutrient load.[107] Calves fed high levels of glucose developed severe diarrhea, with undigested glucose consistently recovered from feces. High levels of glucose also depressed protein digestibility, even when 8 to 10% glucose was added to whole milk. In attempts to increase the energy level of a MR diet, it is possible to exceed enzyme and absorptive capacity of the intestinal tract.

Polysaccharides and other carbohydrates that cannot be digested when fed in MR pass through into the large intestine, where they may then be fermented. Hindgut fermentation may actually create "digestive scours" due to gas production and osmotic pull of undigested carbohydrates and those partially digested by bacterial populations. Bacterial proteins produced in hindgut fermentation are wasted as a source of amino acids. Bacterial proteins produced in the rumen flow into the abomasum and small intestine for digestion and absorption, but bacterial protein produced by large intestine fermentation is simply excreted in feces. This protein loss would decrease calculated apparent dietary protein digestibility unless this bacterial protein fraction could be separated out and subtracted from total fecal protein. Losses would be most problematic when protein sources containing higher polysaccharide content, such as soy flour, are formulated into calf MR.

Lipids

Lipase activates lipolysis to provide free fatty acids for mitochondrial metabolism.[59,182] Salivary and pancreatic lipases in preruminant calves have been analyzed.[182] Salivary lipase behaved in analytical chemical evaluations as a single enzyme with a molecular weight of 52,000, but lipase secreted by the pancreas consisted of 2 distinct enzymes with a molecular weight of 60,000 and 72,000. Colipase is another enzyme secreted by the pancreas, and it is necessary for maximizing lipase activity if bile salt production is impaired or compromised.[15] If the liver secretes adequate bile salts into the lumen of the duodenum, and if adequate emulsification of fat sources has occurred, lipase activity occurs independent of colipase.[136] Lipase secretion from the pancreas was increased or potentiated by proper colostrum administration to the calf immediately following birth.[16,37] Significant increases in lipase secretion occurred when colostrum was administered to calves, and these increases were maintained through weaning and beyond. The importance of pre-duodenal lipase, secreted by salivary glands and gastric glands, is a necessity for efficient intestinal lipolysis of dietary fats.[64]

Micelles of lipids are formed in the intestinal lumen from the product of lipase enzymatic action on triglycerides plus incorporation of 2-monoglycerides, free fatty acids, and bile salts. In preruminant calves, micelles require both 2-monoglyceride and bile salts to form.[169] A more efficient system of micelle formation occurs in older calves which involves lysolecithin, bile salts, and pancreatic phospholipase secretion.[173,185] Fat digestion is complex, with multiple digestive actions required for maximum utilization of dietary fat in the calf. Salivary lipase is a very important contributor to this process.

Intra-gastric lipolysis is important to digestion and absorption of dietary fat. Even when no pancreatic lipase was allowed to interact with digesta, as much as 47% of milk fat entering the ileum was digested to partial glycerides and free fatty acids.[56,57] Calves with full-function pancreatic secretions digested fats more efficiently (60%). Calves absorbed 70% of long-chain triglycerides' fatty acids even when all pancreatic secretions were diverted from the ileum. Combined action of both salivary and pancreatic lipases resulted in efficient digestion and absorption of 96.5% of consumed milk fat.

Nipple-fed calves demonstrated a 3-fold increase in saliva production vs calves drinking from an open top pail, resulting in more salivary lipase entering the abomasum.[194] Simply allowing calves to sham suckle a nipple following a MR meal resulted in a significant increase in measured lipase activity within the abomasum. Two research studies of fat digestibility determined that specific lipolytic activity/gram of digesta was highest in the duodenum, compared to other segments of the gastrointestinal tract.[111,176] Suckling of milk or MR results in liquid bypassing the rumen by means of the esophageal groove. Fats consumed in forage and dry feed are acted upon by lipase produced by rumen microorganisms and salivary lipase swallowed in the process of consumption and rumination. While the measured lipase activity/gram of digesta in the rumen was 20% of that measured in the duodenum, the high volume and mass of digesta in the rumen made rumen digesta, with its accumulated salivary and microbial lipase, a major contributor to the overall lipase activity in ruminating calves.[111]

Alternative Lipid Sources for Calf Milk Replacers

Utilization of coconut oil in calf MR formulations stimulated improved growth rates in preruminant calves.[136] Tallow was compared to coconut oil on fatty acid utilization and oxidation, and on characteristics of intramyofibular and subsarcolemmal mitochondria in heart and skeletal muscles of preruminant calves. Muscle mitochondria, both heart and skeletal, had higher respiratory rates and enzyme activities when calves were fed coconut oil. Feeding coconut oil did not affect palmitate oxidation by intramyofibrillar mitrochondria ($P < 0.05$). Coconut and palm oils are utilized as a fat source in calf MR formulations, but cost may limit their use in the United States.

Irrespective of fat source in calf MR, fat digestibility is considerably increased by homogenization and/or emulsification.[66,151] Emulsifiers are necessary to remove hydrolyzed fatty acids from the interfacial area of fat globules in MR. In weanling piglets, digestibility of soybean and coconut oil was superior to that of tallow ($P < 0.001$), when no emulsification occurred. When tallow was emulsified with lecithin, tallow had superior digestibility ($P < 0.007$).[66] Homogenizing tallow and lard fat sources with skim milk or whey proteins, plus emulsification with lecithin and commercial emulsifiers, are common methodologies used for fats added to MR in the United States.[200] This process reduces micelle size of the fat source and surrounds it with polar proteins. This makes it possible for the protein-encapsulated fat globule to be suspended in warm water for feeding. This prevents the fat globules from rising to the top of the MR mixture like unhomogenized cream rises to the top of cooled whole milk.

Lipid research has determined what type of fat source will optimize calf performance or produce performance comparable to that of milk fat. Highly unsaturated vegetable oils fed in MR produced an increased incidence of diarrhea and poor weight gains.[1,2,10,60,80,81,82,84] Vegetable oil digestibility can be improved with hydrogenation, emulsification, and homogenization.[82,83,150,151] Corn-oil-induced calf diarrhea can be tempered by proper homogenization to reduce fat globule size to 1 μm, compared to calves consuming corn-oil globules of 10 to 20 μm.[82] The impact of salivary lipase on various vegetable oils and animal fats was measured as they were mixed with lecithin, (80% fat to 20% lecithin by weight), then mixed into dried skim-milk (10% fat-lecithin mix to 90% dried skim-milk), and passed through a crude homogenizer (unknown fat globule size produced).[165] Pregastric lipase was collected from an esophageal cannulated 1-month-old calf, sham-fed non-fat MR through a nipple. Digesta were collected, filtered of mucoid saliva, and utilized to incubate individual samples of MR containing the different fat sources. Milk fed by suckling from a nipple had significant products of fat digestion when sampled, with substantial amounts of di- and mono-glycerides. When milk was placed directly into the abomasum, undigested triglycerides predominated in sampling, regardless of time after feeding (0.5, 1, or 2 hours post-administration), and undigested triglycerides were 25 to 35% greater than those discovered in nipple-fed calves.[165]

Butter fat, butter oil, and coconut oil contain considerable quantities of short-chained fatty acids, whereas lard, tallow, and soybean oil contain only small or very small amounts of short-chained fatty acids.[131] Relative activity of pregastric lipase for butter fat, colostral fat, and coconut oil averaged 81.8 compared to the activity measured for evaporated milk. Average relative activity for choice white pork grease, refined lard, tallow, soybean oil, and corn oil was 19.6. The reduced activity for salivary lipase on choice white pork grease, refined lard, tallow, soybean oil, and corn oil was thought to be due to these lipid sources lacking in short-chained fatty acids.[59,146,147,165] The authors reported salivary lipase does not hydrolyze fats lacking short-chained fatty acids.[59,165] Since maximum pancreatic lipase secretion does not occur until the calf is 8 days of age, salivary lipase becomes a very important contributor to the calf's digestive physiology for lipid digestion.[155]

Calves under 3 weeks of age can adequately digest up to 2.5 grams of fat/lb (5.4 grams/kg)/day.[121] A typical US MR powder has 20% crude fat on an as-is basis. Feeding 1.25 lb (0.56 kg)/day of this formulation to a 100 lb (45.4 kg) calf provides the calf with 114 grams of fat, less than half the 248 grams allowed by these findings. When various fats were fed to preruminant calves at 20% of MR powder on an as-is basis, butterfat had an apparent digestibility of 97%; lard, 96%; palm oil, 95%; and tallow 90.4%.[155]

Highly unsaturated vegetable oils are reported to produce diarrhea and reduced weight gains when compared with mostly saturated forms of animal fat.[1,2] Industrial hydrogenation of unsaturated vegetable oils improves their digestibility and utilization.[151] Mechanical homogenization further improved utilization of fat sources by neonatal calves.[82,84,145] Low-pressure homogenization, producing fat globules of various diameters, provided superior performance if fat content of MR powder was formulated to provide more than 20% fat on a dry-matter basis.[83] Calves consuming lard as the fat source in a MR powder produced significantly greater feed conversion, but a mixture of palm, coconut, and rapeseed oils, and a mixture of palm and coconut oils, produced similar health and body weight gain, compared to calves consuming a lard-formulated MR.[90] This study and others confirmed that properly manufactured and emulsified vegetable oils could produce acceptable performance when used as a fat source for the neonatal calf.[74,150,151,154,184]

Use of Alternative Proteins and Long-term Effects of Accelerated MR Feeding on Milk Production

Little peer-reviewed research is available that describes the outcomes from feeding alternative ingredients in an accelerated feeding program for replacement dairy heifers. There is a considerable volume of data published by MR manufacturers, but it has not been peer-reviewed. Utilization of accelerated feeding programs commonly results in the feeding of 2.5 lb (1.1 kg) of MR powder/calf/day in an 8-quart (7.6 L) volume, exactly twice the dry matter intake of traditional MR feeding regimens. More peer-reviewed data are required to determine the impact of these higher feeding rates of alternative ingredients on the health and performance of replacement dairy heifers.

Four recent research studies are useful in considering alternative proteins in accelerated heifer development nutritional programs. In the first study, 2 inclusion rates of SWGP were used with addition of essential amino acids in a 28.5% CP, 15% fat MR fed at 1.32 lb (0.60 kg)/day during weeks 1 and 2; 1.76 lb (0.80 kg)/day during week 3; 2.64 lb (1.20 kg)/day in weeks 4 through 6; 1.76 lb (0.80 kg)/day in week 7; and 1.32 lb (0.60 kg)/day during week 8.[79] All formulas contained the same 36% inclusion rate of skim milk and all calves were introduced to starter grain (21.8% CP) at 28 days of age. Whey-protein concentrate was used to supply additional protein beyond the standardized skim-milk contributions in the all-milk protein-based formulas, while SWGP was used at 4.5% or 9.0%, replacing WPC in the formula and contributing 21% or 42%, respectively, of the total protein in the formula. ADG and body dimensions at 56 days and 90 days did not differ among treatments. Further, there was no effect on observed health variables.

The second trial examined spray-dried bovine plasma (SDBP) in a 20% fat, 22% CP, and 2% lysine MR at either 5% or 10% inclusion, and either with only additional synthetic methionine to match the amino acid profile of the all-milk control MR or with additional synthetic methionine, isoleucine, and threonine to match the all-milk control MR. MR (12.5% solids) was limit-fed to Holstein bull calves twice daily at 10% of body weight on days 1 and 2, 12% of body weight on days 3 through 7, and 14% of body weight during days 8 through 35, adjusted weekly.[120] Average daily MR intake ranged from 1.79 to 1.83 lb (0.81 to 0.83 kg), depending on the average calf body weight of the treatment group. No calf starter was fed. ADG was not different between groups, but tended ($P = 0.08$) to be lower for calves fed MR containing 10% plasma with only added methionine. Calves fed plasma were healthier: calves fed all-milk MR were 3.55 times ($P < 0.001$), 3.39 times ($P = 0.0002$), and 2.48 times ($P = 0.001$) more likely to be administered antibiotics than calves fed MR containing 5% plasma plus methionine, 5% plasma plus methionine, isoleucine and threonine, or 10% plasma plus methionine, isoleucine and threonine, respectively. Calves fed all-milk MR tended to have ($P = 0.07$), or had a greater incidence ($P = 0.01$) of scours compared to calves fed MR containing 10% plasma with added methionine or MR containing 10% plasma with added methionine, isoleucine, and threonine, respectively.

Two more recent research trials in veal calves discovered that the addition of SDBP resulted in similar[197] or improved[198] performance compared to an all-milk formula when fed in a strategy that had many similarities to an accelerated MR feeding regimen. In the first of 2 trials, sale-barn sourced Holstein bull calves were fed 148 lb (67.1 kg) of MR solids from placement in the barn until day 56.[197] No grain or forage was fed. Calves were fed a 17% CP, 19% fat all-milk MR, supplemented with either spray-dried whole colostrum, or an isonutritious blend (50% CP and 20% fat) of encapsulated fat and either SDBP or whey protein concentrate (WPC) that was isonutritious (50% CP and 20% fat) to colostrum. Each respective supplement was added to individual feeding bowls at 0.22 lb (0.1 kg)/calf/day for 42 days, and stepped down to withdrawal at 56 days. The combined intake of MR and supplement at calf placement was 0.63 lb (0.29 kg) of what was calculated to be a 25.8% CP, 19.8% fat diet; the feeding rate gradually increased to 2.94 lb (1.33 kg) of a 20% CP, 19% fat diet by day 28, and 4.2 lb (1.90 kg) of an 18% CP, 19% fat diet by day 56. Total plasma intake was 4.5% of MR consumption. One-half as many calves were treated during the peak scour period (week 1) in plasma- or colostrum-supplemented calves vs whey protein concentrate ($P < 0.05$). Plasma-supplemented calves also received fewer antibiotic treatments ($P < 0.05$). No differences were noted in 56-day ADG, and calves gained 113% and 115% of their arrival weight by 56 days for plasma- and WPC- or colostrum-supplemented calves, respectively. In the second of 2 veal-calf trials, sale barn-sourced Holstein bull calves were fed 129.3 lb (58.6 kg) of MR solids from placement until 53 days.[198] Again, no grain or forage was fed. All calves were fed a 17% CP, 19% fat all-milk MR that had an additional 5.2% of total solids added as SDBP. In addition to SDBP added to the diets of all calves, calves were supplemented in their individual feed bowls with either additional spray-dried whole colostrum (13% IgG) or a blend of 70% SDBP and dry-encapsulated fat that was estimated to be similar in IgG to colostrum, or a blend of WPC and encapsulated fat that was isonutritious to the spray-dried whole colostrum. Each respective supplement was added to individual feeding bowls starting at 1.76 oz (49.93 g)/day and slowly decreased to just 0.16 oz (4.54 g)/day by 53 days. Each calf received 2.5 lb (1.13 kg) of respective supplement by 53 days. Total diet was approximately 0.75 lb (0.34 kg) of 25% CP and 18% fat MR at placement, 1.92 lb (0.87 kg) of 21% CP and 18.2% fat MR at 21 days, and 3.84 lb (1.74 kg) of 20% CP, 18% fat MR at day 53. At 53 days, calves gained 103.4%, 97.7%, and 100% of their placement body weight for plasma-, WPC- and colostrum-supplemented groups, respectively. Calves fed extra plasma outgained ($P < 0.031$) those supplemented extra WPC. Colostrum provides 90 to 95% IgG_1,[24] and bovine plasma provides 50% IgG_1 and IgG_2.[23] The IgG provision by WPC mirrors that of colostrum, but in much-reduced quantities.[25,26] Mean IgG_1 in 349 individual milk samples was 0.46 mg/mL and mean IgG_2 in 355 milk samples was 17 ug/mL. Irish researchers determined the IgG content of 35% CP WPC was 1.5% w/w. At 34% CP, WPC would provide 2.27 grams of IgG/lb (0.45 g) of WPC.[47]

A review of accelerated feeding practices for replacement dairy heifer calf development has been published.[89] While potential benefits of accelerated milk-replacer feeding were attributed to reduced age at first calving, recent analyses[170] indicated that benefits may accrue to subsequent milk yield. Ten years of calf-growth data and subsequent first-lactation (1,244) records at the Cornell University research dairy herd were evaluated from calves in which greater than 90% were fed a 28% protein and 15% fat MR. A Test Day Model was developed utilizing inputs of preweaning daily gain, birth weight, weaning weight, calving age, birth year, birth month, and calculated energy intake over estimated maintenance requirements. Another NY herd also had 623 first-lactation records available over a 5-year period, and all calves were also fed the same 28:15 MR. For every additional 1 lb (0.45 kg) of daily gain (within the range of 0.22 to 3.5 lb (0.10 to 1.59 kg)/day), heifers produced 850 lb (385.6 kg) more milk during their first lactation ($P < 0.01$) and produced 2280 lb (1034.2 kg) more during their first 3 lactations (the NY herd had a 30% greater response). An additional 235 lb (106.6 kg) more milk was produced in the first lactation and 1990.7 lb (903 kg) more milk was produced over 3 lactations for every Mcal metabolizable energy intake above maintenance during the pre-weaning period. Calves born during winter months (average temperature of 32 °F (0 °C)) consumed an average of 1.43 Mcal/d less energy above maintenance than calves born during warmer months (average daily temperature of 67 °F (19.4 °C)). Preweaning daily gain accounted for 22% of variation in first-lactation milk yield. Age at first calving did not affect milk production within a range of 20 to 30 months. Colder weather for calves negatively affected subsequent milk production, as less energy was available over increased maintenance needs for young calves, resulting in their lower growth rate. Probable mechanisms for this increased milk yield are not understood, but are speculated to be related to very early mammary gland development. This may be due to epigenetics whereby better nutrition and bioactive factors turn on more genes, which subsequently result in more milk in subsequent lactations of those calves fed on an accelerated plane of nutrition. Bioactive factors are contained in greatest concentrations in colostrum,[18] but there also are lower levels in transition milk, regular milk, and in the various milk by-products used in calf milk replacers.[170,171] Other studies, which measured subsequent first-lactation milk production as related to pre-weaning performance, have generally found positive effects, but animal numbers were too few to develop the Test Day Model noted above and achieve statistical significance. In practical terms, the response in increased subsequent milk production is related to increased rate of daily gain, without undue fattening, during the first 2 months of life of the calf.

Summary and Conclusions

Plant Proteins

- Plant-based proteins (and carbohydrates and oils) are widely used in the European milk replacer industry, for both veal and dairy heifer calf MR formulas.
- When replacing 15 to 20% of the milk protein in the MR formula, SWGP will perform comparably to milk protein in conventional (1.2 lb (0.54 kg)/d MR powder) MR feeding strategies; however, results are not consistent as several trials demonstrated relatively poorer performance.
- Improperly or minimally processed soy or pea products result in profound intestinal damage, reduced enzyme production, relatively poorer digestibility, and reduced daily gain in comparison to all-milk formulas. Antigenic proteins are absorbed and such products are not suitable for use in MR. To insure high digestibility, soy products should be void of β conglycinin, be extremely low in antitryptic activity and ∞-conglycinin, and have low polysaccharide content.
- Four quarts (3.79 L) of colostrum, measuring 22-23 BRIX, administered as soon after birth as possible, improves gastrointestinal tract development, enzyme production, and nutrient uptake.

Animal Proteins

- Spray-dried plasma protein fed at inclusion rates of up to 7.5% of the absolute MR formula produces performance comparable to milk protein, and in

disease-challenged calves the addition of plasma to MR reduces mortality and morbidity, while also improving daily gain and starter grain intake.
- Disease challenge trials (*Cryptosporidium parvum*, bovine coronavirus, and *E. coli*) demonstrated improved performance and health of calves when plasma-based products were added to MR.
- Spray-dried egg protein in MR has an upper limit of inclusion, which is variable depending on the source.

Carbohydrates
- The calf has limited capacity to effectively utilize lactose replacements in MR.
- Corn-syrup solids or dextrose can be included at rates of up to 10% of the absolute formula with no negative effects on growth; however, incidence of scours may increase. Glycerin can be added at even higher rates with no negative impacts on performance.

Lipids
- Lipid digestion is complex, requiring salivary lipase, bile salts, and various pancreatic secretions to efficiently digest and absorb as much as 96 to 97% of ingested milk fat, 96% of lard, 90.4% of tallow, and 95% of palm oil. Calves less than 3 weeks of age can adequately digest up to 2.5 grams/lb (5.4 g/kg) of body weight daily, meaning a 100 lb (45.4 kg) calf can consume up to 248 grams (about one-half pound) of properly processed MR fat on a daily basis.
- Fats and oils should be properly homogenized and/or emulsified by using commercial emulsifiers and lecithin. These practices improve digestion.
- Highly unsaturated vegetable oils, such as soybean and corn oil, are poorly utilized by the calf, and should be avoided. Industrial hydrogenation improves their utilization.
- Short-chain fatty acids increase salivary lipase secretions. Maximum pancreatic lipase secretion does not occur until the calf is 8 days of age. Butter fat, butter oil, and coconut oil contain considerable quantities of short-chain fatty acids, while lard and tallow do not.

Alternative MR Ingredient Recommendations

This review indicates that MR containing 5 to 7% SDBP or a combination of 5% SWGP and 5% SDBP yields performance similar to all-milk-protein MR. If a plant-based protein is used alone, SWGP at 5% in the second of a 2-stage MR feeding strategy consistently performs comparably to an all-milk-protein MR. All examples are based on a percentage of the absolute MR formula provided by the respective alternative protein ingredient. Amino acids should be properly balanced.

Limit the use of lactose replacements to 5 to 10% of the absolute formula.

Coconut oil may improve growth rate and should be considered for use at 20 to 25% of the lipid composition. Avoid the use of highly unsaturated oils like soybean oil.

Palm oil can effectively replace lard or tallow in MR. Homogenized and emulsified lard and tallow are used extensively as the primary lipid source in MR.

Accelerated MR Feeding and Alternative MR Ingredients

At this time there is little data available from which to draw valid conclusions. The much higher feeding rates of MR powder in accelerated feeding programs may impact the quantity and type of alternative ingredients formulated into MR for a source of protein and/or fat. The impact of higher feeding rates of these alternative ingredients on health and performance of calves fed higher quantities of MR powder needs further evaluation.

Accelerated feeding programs have merit, as a 10-year data base with 1,244 calf and subsequent cow records has demonstrated for every 1 lb (0.45 kg) of daily gain (within the range of 0.22 to 3.5 lb (0.1 to 1.59 kg)/day) during the first 2 months of life, heifers produced 850 lb (385.6 kg) more milk during their first lactation and 2,280 lb (1034.2 kg) more during their first 3 lactations. Preweaning daily gain also accounted for 22% of variation in first-lactation milk yield. Most of these data were accrued from calves fed a 28% CP and 15% fat MR at much higher rates than traditional (1.2 lb(0.54 kg)/day MR powder) calf feeding programs.

Endnote

[a]Karo® Syrup, ACH Food Companies, 6400 South Archer, Oakbrook, IL

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Product and Laboratory List

- FP1000 hydrolyzed wheat gluten protein, MGP Ingredients, Cray Business Plaza, 100 Commercial St., Atchison, KS 66006
- Nutrior hydrolyzed wheat gluten protein, Chamtor, Les Schettes – CS 30004, 51100 Bazancourt Cedex, France
- HP 100 soy protein concentrate, Hamlet Protein, Saturnvej 51, 8700 Horsens, Denmark
- Archer Daniels Midland Europoort B.V. (ADM), Elbeweg 139, 3198 LC Europoort-RT, P.O. Box 1105, 3180 AC Rozenburg, The Netherlands
- Nutrapro spray-dried animal plasma, APC, 2425 SE Oak Tree Court, Ankeny, IA 50021
- Proliant 8000, Proliant Dairy Ingredients, 2425 SE Oak Tree Court, Ankeny, IA 50021

- Whey Protein Concentrate 34%, Milk Specialties Global Animal Nutrition, 7500 Flying Cloud Drive, Suite 500, Eden Prarie, MN 55344
- LifeLine,™ APC, 2425 SE Oak Tree Court, Ankeny, IA 50021
- Laboratory to test soy products or MR for β-conglycinin: TNO Triskelion BV, Utrechtseweg 48, P.O. Box 844, 3700 AV Zeist, The Netherlands. Attn: Ria van Biert or Gert van Duijn. E-mail: gert.vanduijn@tno.triskelion.nl

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